

Diet, microparticles and atherothrombosis

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1. ABSTRACT

Cardiovascular disease is the main cause of death worldwide, and is principally caused by atherosclerosis, with subsequent thrombus formation, eventually provoking an acute myocardial infarction or a stroke. The formation and progression of the atherosclerotic plaques responds to multiple factors including certain diets. Intensive research has elucidated the role of diet in cardiovascular disease and has led to public health policies focusing on educating the population on the role of nutrition in cardiovascular health. Compelling evidence shows that a healthy diet,

rich in fruit and vegetables with moderate consumption of fish and a low consumption of animal by-products and processed foods, decreases low-grade inflammation and oxidation, leukocyte activation, platelet aggregation and microparticle shedding. Thus, following such a diet decreases the incidence of cardiovascular disease, lowers mortality and delays the progression of atherothrombosis. Identifying novel risk factors for cardiovascular disease and understanding how food impacts on the disease helps in the development of novel preventive measures.

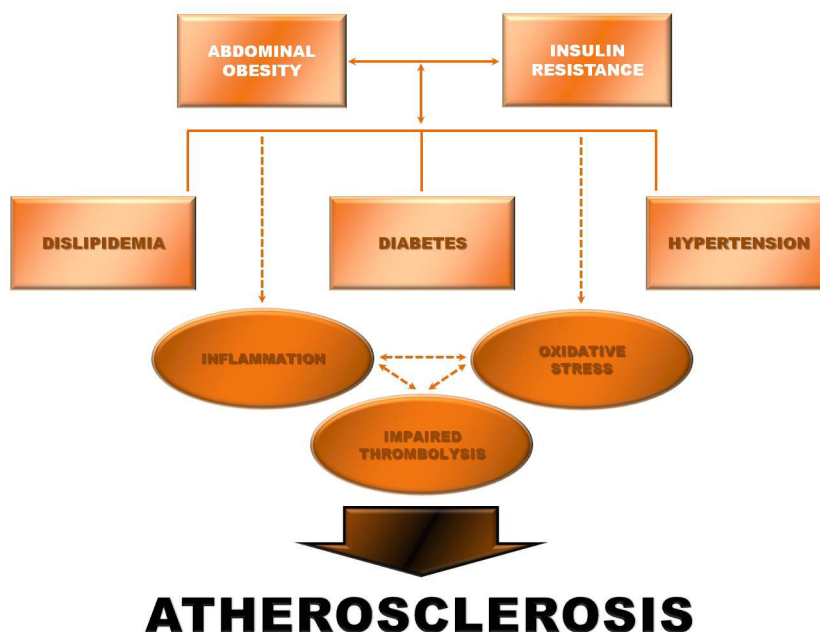


Figure 1. Cardiovascular risk factors leading to atherosclerosis. Central adiposity and insulin resistance play key roles in the development of dyslipidemia, diabetes and hypertension, which in turn, cause endothelial dysfunction by increasing low-grade inflammation and oxidation and impairing the thrombolytic capacity, thus favoring the onset and progression of atherosclerosis.

2. INTRODUCTION

Cardiovascular disease (CVD) is the main cause of death worldwide. In 2012 it caused 17.5 million deaths, which accounts for more than 30 percent of total mortality, and more than 46 percent of non-communicable diseases (1). Principally, it is caused by the formation of atherosclerotic lesions in the inner layer of the artery with the subsequent thrombus formation that eventually obstructs an artery thus provoking a major adverse cardiovascular event (MACE), namely an acute myocardial infarction (AMI) or a stroke. The formation and progression of the atherosclerotic plaque responds to multiple factors that will be discussed from this point forward.

In recent decades, there has been intensive research to elucidate the role of diet in CVD in order to establish sustained public health policies giving nutritional advice to the general population. Nevertheless, the study of the relationship between diet and CVD is extremely complex for several reasons. Although necessary, the study of the effects of isolated molecules (i.e. vitamins, fatty acids (FA) or polyphenols) does not take into account the effect of the food matrix in which it is involved, that it may also contain other molecules than can interfere (by stimulating or inhibiting) with its absorption or activity. On a larger scale, the same happens with the study of a single food, where effects may differ according to the whole dietary pattern. Another factor adding complexity to the relationship between diet and CVD

is the time of exposure to nutrients, foods and dietary patterns. Because of evident logistical, technical and economical reasons, it is very difficult to study the long term effects of diet in CVD, and therefore, very few long term interventional studies have been performed. In light of this, this review aims to update current knowledge on the complex relationship between diet and atherothrombosis.

3. THE ATHEROSCLEROTIC PROCESS

Atherosclerosis is a chronic oxidative and inflammatory disease of the large arteries that is initiated at early stages of life by progressive deposition of lipids in the intima layer. Indeed, current evidence suggests that obesity in childhood increases metabolic syndrome (MetS) rates, subclinical CVD (2), and overall CV risk of mortality in adulthood (3–5). During lifetime, it can develop at a higher or lower degree depending on the CV risk burden accumulated. In this line, the American Heart Association strategic goal for 2020 and beyond is focused on maintaining CV health from birth and during life time (6).

This section reviews the main risk factors contributing to atherosclerosis development.

3.1. Risk factors for atherosclerosis

The relationship between obesity, insulin resistance and atherosclerosis is depicted in Figure 1. The main triggers of atherosclerosis are abdominal obesity and insulin resistance (7–8). Increased visceral

fat is associated with a shift in the normal balance of the adipokines resulting in a pro-inflammatory state thus promoting insulin resistance and vice versa. Both factors contribute to the development of diabetes, dyslipidemia and hypertension, overall conforming the MetS or syndrome X (9), through the interaction between immune and endothelial cells. These subclinical situations are independent risk factors but their presence promotes the initiation of others, an underlying sustained oxidation and inflammation, and reduced nitric oxide availability, resulting in endothelial dysfunction (10), impairing thrombolysis and increasing the procoagulant state, synergistically amplifying atherothrombosis and thus increasing the risk of MACE.

3.1.1. Diabetes

Diabetes is characterized by insulin resistance and chronic hyperglycemia. Both insulin resistance and hyperglycemia promote inflammation (11) and endothelial dysfunction by increasing reactive oxygen species (ROS) generation and reducing nitric oxide (NO) production (12). Therefore, the development of vascular inflammation and oxidative stress are two key mechanisms involved in endothelial dysfunction and atherosclerosis progression in diabetic patients (13,14).

3.1.2. Obesity and the metabolic syndrome

Obesity is defined as a body mass index over 30 Kg/m². Overweight and obesity were estimated to account for 3.4 million deaths and 93.6 million disability-adjusted life-years in 2010 (15,16). Obesity increases the risk of developing diabetes, hypertension, coronary heart disease, stroke, certain cancers, obstructive sleep apnea and osteoarthritis (17).

MetS is a pathological condition defined by a low-grade inflammation and characterized by the cluster of three or more independent CV risk factors, namely abdominal obesity, hyperglycemia/insulin resistance, hypertriglyceridemia, low high-density lipoprotein (HDL) and/or high low-density lipoprotein (LDL) cholesterol, and high blood pressure (18). Accumulation of these cardiometabolic risk factors has been associated with increased CVD (19) and all-cause mortality (1). The prevalence of both obesity and type 2 diabetes has increased dramatically in recent decades worldwide. Both conditions represent substantial risk factors for the development of atherosclerotic disease and the resulting increased incidence of myocardial infarction and stroke (20).

3.1.3. Hypertension and endothelial dysfunction

Hypertension is an asymptomatic disease caused by elevated blood pressure levels. One of the principal effects of hypertension is vasoconstriction and

impaired NO availability (21) triggering inflammation and endothelial dysfunction (22), again increasing CV risk (23,24).

3.1.4. Dyslipidemia

Dyslipidemia is the elevation of triglyceride blood levels and elevation of LDL and/or reduction of HDL cholesterol blood levels. FA as well as other components of triglycerides and LDL are subject to oxidation and lead to the development of oxidative stress and accumulation at the inner layer of the blood vessel (25). Besides the accumulation of LDL particles at the endothelium, hyperlipidemia increases the production of ROS, which damage the endothelium promoting atherogenesis (15).

Overall, primary prevention of CVD is the first-line revetment to minimize the impact of CVD and its comorbidities, considering that CV risk factors are interrelated and act synergistically. Thus, CVD prevention involves control and reduction of CVD risk factors and maintenance at physiological ranges in order to delay the onset and development of atherosclerosis and finally MACE presentation, as pinpointed in current guidelines for the prevention of CVD from different international societies (26–28).

3.2. Atherosclerosis and thrombosis

Atherosclerosis is the progressive narrowing of arteries. It is initiated by the accumulation of LDL cholesterol particles in the sub-endothelial space of the arteries (intima layer) and the change of phenotype in the overlying endothelial cells that will induce recruitment and transmigration of monocytes. The internalized monocytes will differentiate to macrophages that when overloaded with lipids will transform into foam cells accumulated in the artery wall forming the fatty streaks, the precursor of the atherosclerotic plaque. Figure 2A depicts the early stages of atherosclerosis. When increased levels of LDL-cholesterol are maintained over time, LDL particles become trapped in the artery, undergoing progressive oxidation, and activate endothelial cells leading to expression of chemokines and adhesion molecules that trigger innate immunity response and attract circulating monocytes to the vessel wall (29). In this process, modified LDL particles are internalized by intimal monocytes, where they differentiate into macrophages and become foam cells as the result of accumulation of lipid droplets within the cytoplasm (30), and secrete pro-inflammatory cytokines and reactive oxygen species that amplify the local inflammatory and oxidative response at the injury site. Macrophages and foam cells accumulate in the core of the atherosclerotic plaque where they undergo apoptosis and necrosis, forming the proinflammatory and thrombogenic necrotic core (25).

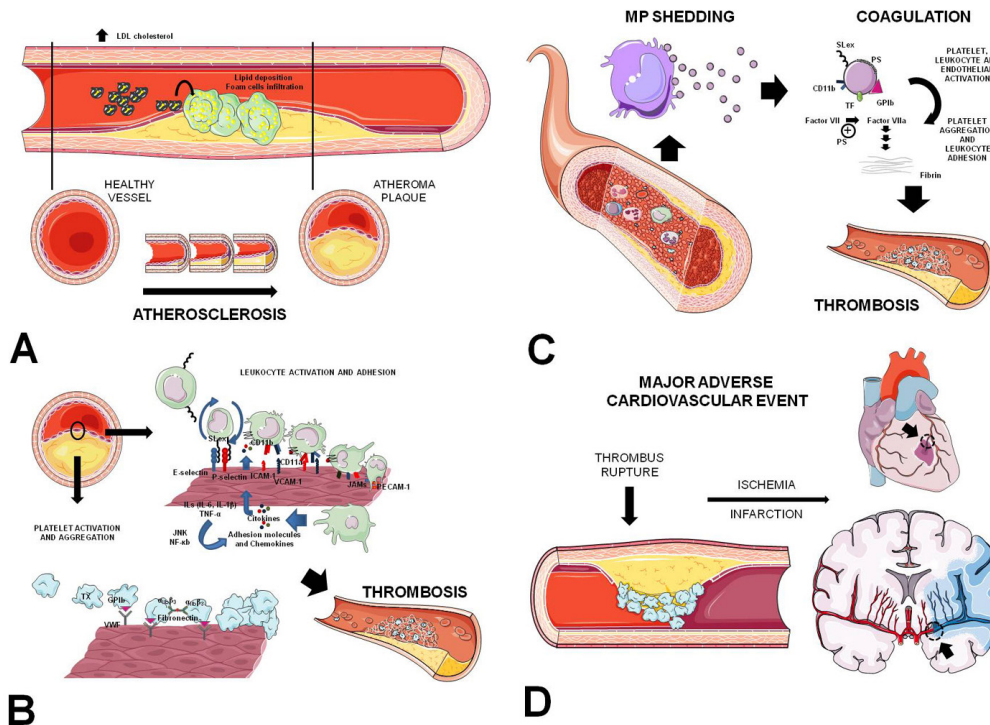


Figure 2. Schematic representation of the atherothrombotic process. A) At the initial steps of atherogenesis, LDL cholesterol molecules deposit into endothelial cells, at the inner wall of the artery where they are oxidized. Macrophages move into the vessel wall in an attempt to remove the excessive lipid deposition provoking their shift to foam cells, while the plaque continues to grow. B) The oxidation of LDL and foam cell activity provokes endothelial dysfunction and the release of proinflammatory cytokines and adhesion molecules that recruit leukocytes at the atherosclerotic sites where they transmigrate through the intima layer. This is accompanied with platelet activation, platelet-platelet adhesion and aggregation at the endothelial wall thus forming a stable thrombus. C) As a consequence of leukocyte and platelet activation, and simultaneously to the release of cytokines and adhesion molecules, platelets, leukocytes and endothelial cells release circulating microparticles, which contain phosphatidylserine (PS) in their surface, that with tissue factor (TF) stimulate the production of fibrin and thus, feeding back the thrombotic process. D) Eventually, the thrombotic core of the plaque breaks by erosion or chemical activation and the thrombus obstructs an artery provoking a major adverse cardiovascular event such as an acute myocardial infarction or a stroke.

Atherosclerosis has long been considered an oxidative disease (Figure 2A) but it also involves low-grade chronic inflammation (Figure 2B-2C). As will be further discussed, in its early stages atherosclerosis involves endothelial activation triggering platelet and leukocyte activation, adhesion and trans-endothelial migration to the intima (29). Inflammation is characterized by a complex orchestra of molecular and cellular signals that cause several reactions in the injured area and, over prolonged periods of time, these lipid-laden cells are more prone to apoptosis, which further contribute to increased plaque size and vulnerability, making them more likely to rupture, with ensuing thrombosis, finally leading to a MACE.

3.2.1. Leukocyte activation and platelet aggregation

Infiltration and modification of LDL particles and foam cell formation causes endothelial dysfunction and the release of proinflammatory cytokines from endothelial cells that promote the recruitment of leukocytes, mainly T-lymphocytes (31)

and monocytes/macrophages (32) to the endothelium and the subsequent infiltration, increasing plaque instability.

Monocytes differentiate into macrophages and take up the modified LDL in an unregulated fashion eventually leading to the formation of foam cells which further perpetuate local inflammatory responses and cell recruitment. Macrophages are the most numerous leukocyte subpopulations within the atherosclerotic plaque probably because of their role in lipid uptake. As the lesion develops, endothelial cells are activated through pro-inflammatory stimulus, and they increase their expression of various leukocyte adhesion molecules, such as VCAM-1, ICAM-1 or E-Selectin. Once adherent to the activated endothelial layer, leukocytes (mainly monocytes, B and T lymphocytes) enter to the intima layer influencing cells already present in the atheroma plaque, amplifying the pro-inflammatory status by secreting inflammatory cytokines and growth factors, and thus creating a vicious circle to further stimulate macrophages, as depicted in Figure 2B.

Figure 2B also schematizes platelet activation and aggregation that occurs during, and contributes to plaque formation in the early stages of atherogenesis. During this process, platelets become activated upon binding to collagen and von Willebrand factor (vWF) with their surface receptors, which are secreted in response to the endothelial inflammatory stimulus from damaged endothelial cells. The activated platelets then secrete a range of adhesion molecules, such as P-selectin and CD40 ligand, forming platelet-leukocyte aggregates and provoking adherence to the endothelium (33), thus promoting atherosclerosis progression. Additionally, platelets bind fibrinogen from plasma and they synthesize and secrete agonists such as adenosine diphosphate (ADP) and thromboxane A₂, which also induce platelet aggregation and thus amplify and maintain the initial platelet response. Platelet aggregation occurs in parallel with the formation of platelet-monocyte aggregates. Platelet-monocyte aggregates are sensitive markers of platelet activation and contribute to the initiation and progression of atherosclerosis (34). After atherosclerotic plaque rupture, there is a feedback loop of systemic inflammation at injury sites mediated by platelets and leukocytes (35), perpetuating the atherosclerotic lesion.

3.2.2. Thrombus formation

As depicted in Figure 2A-D, the pathogenesis of MACE is primarily driven by atherosclerosis, thrombosis and cardiac or brain injury, with subsequent deterioration in organ function. Heart attacks and strokes are usually acute events and are mainly caused by artery occlusion that prevents blood from flowing to the heart or brain (Figure 2D), resulting in ischemia, which in turn leads to the release of damage-associated molecular patterns, thus resulting in necrosis and/or tissue repair (36).

Atherosclerosis is the major underlying cause of MI and stroke. Platelets circulate in resting state, and after atherosclerotic plaque rupture, platelets are activated and aggregated by intraplaque components such as tissue factor, vWF and collagen, or by soluble platelet agonists (thrombin, ADP or thromboxane A₂ formed from activated platelets), which promote thrombus formation (Figure 2B). The initial tethering is mainly mediated by platelet glycoprotein Ib/alpha receptor and endothelial vWF, anchored to collagen. Activated platelets then further release thrombin and ADP, which act on platelet P_{2Y}₁₂ ADP receptors and has a central role in amplifying the response of platelets to the initial stimulus (29). Platelet aggregation occurs by mediation of integrins (alpha IIb beta 3) and protein disulfide isomerase (37,38) and leads to the activation of the coagulation cascade and the formation of a stable cross-linked fibrin clot (Figure 2B-D). Another key factor in thrombosis is

tissue factor, a small transmembrane glycoprotein. Tissue factor binds Factor VIIa, and this complex converts Factor X to Factor Xa, leading to thrombin generation and fibrin formation. Tissue factor pathway inhibitor (TFPI) inhibits this pathway. Tissue factor is found sequestered within atherosclerotic plaques, and plaque rupture allows tissue factor exposure to the circulation, leading to formation of a thrombus (39–42). In addition, collagen exposure after plaque rupture has a pivotal role in thrombus formation by maintaining platelet adhesion to endothelium through glycoprotein Ia/IIa and by activating the conversion of Factor XII to Factor XIIa leading to thrombin generation and fibrin formation (43). The balance between prothrombotic factors and endogenous fibrinolysis determines whether the thrombus grows, propagates or instead proceeds to its dissolution (44). Advanced lesions at risk of rupture are characterized by accumulation of diseased vascular cells and apoptotic cellular debris (45), and why these cells are not cleared within atheroma remains unknown. Therefore, platelet activation, aggregation, and the subsequent generation of an occlusive intra-arterial thrombus are essential steps in atherothrombotic disease, and enhanced platelet reactivity is associated with both the extent of end organ injury and adverse prognosis (46).

Besides the role of platelets, monocytes and lymphocytes in atherogenesis, neutrophils may also play a significant role in the progression of atherosclerosis and thrombus formation. This leukocyte subset is recruited at the site of plaque rupture where they generate extracellular traps (called neutrophil extracellular traps -NETs-), composed of a chromatin network with granular and cytoplasmic proteins that eventually expose tissue factor inducing thrombin generation (47), and thrombosis (48).

3.3. Circulating microparticles

According to the International Society of Thrombosis and Haemostasis, microparticles (MPs, also referred as microvesicles) are 0.1 to 1.0 µm membrane blebs originated from almost all types of cells (49). MPs are formed when cells are activated or undergoing apoptosis (50), thus reflecting the state of the cell from which they are originated and may be potential useful biomarkers for various disease processes. Indeed, there is an increasing interest in MP quantification in the context of several diseases such as cardiovascular diseases, cancer or certain hematological disorders.

Several MPs have been found in plasma samples, and platelet-derived circulating MPs (cMPs) are believed to be the most abundant (51,52), at least in healthy individuals. cMPs act as procoagulant agents because an externalization of the procoagulant anionic phosphatidylserine takes place during MP formation.

Table 1. Cell molecules most widely used for circulating microparticle identification and characterization

mAb	Alternative name	Expression
AV	PS-binding protein	Widely expressed
CD61	beta ₃ -integrin	Platelets
CD41	Integrin alpha-IIb	Platelets
CD142	Tissue Factor	Platelets, monocytes, macrophages, EC, SMC, fibroblasts
CD62P	P-Selectin	Platelets, Endothelial Cells
CD146	Melanoma Cell Adhesion Molecule	Endothelial Cells
CD144	Vascular endothelial cadherin	Endothelial Cells
CD62E	E-Selectin	Endothelial Cells
CD309	VEGFR-2	HSC, EPC and EC
CD31	PECAM-1	Leukocytes, platelets, EC
CD42b	Glycoprotein Ib alpha chain	Platelets and megakaryocytes
CD235ab	Glycophorin A and B	Erythrocytes
CD3	T-cell co-receptor	T-Lymphocytes
CD45	Leukocyte Common Antigen	Leukocytes
CD14	LPS-receptor	Macrophages, monocytes
CD62L	L-Selectin	Leukocytes
CD11b	Macrophage-1 Antigen (Mac-1)	Neutrophils, leukocytes

mAb indicates monoclonal antibody; AV, annexin V; CD, cluster of differentiation; PS, phosphatidylserine; VEGFR-2, vascular endothelial growth factor receptor-2; PECAM-1, Platelet endothelial cell adhesion molecule; LPS, lipopolysaccharide; SMC, smooth muscle cells; HSC, hematopoietic stem cells; EPC, endothelial progenitor cells; and EC, endothelial cells.

Phosphatidylserine exposure on MP surface enhances platelet adhesion to the endothelium, provides binding sites for coagulation enzymes assembly, and induces tissue factor activity, thrombin generation and thrombus formation (53) (Figure 2C). In addition, cMPs also contain bioactive molecules from their parental cells that feed back platelet, leukocyte and endothelial cell activation reinforcing the atherothrombotic process. Additionally, cMPs can contain tissue factor in its surface, stimulating fibrin generation as previously explained. Indeed, *in vitro* studies demonstrated that increased levels of cMPs promoted plated deposition on the arterial wall (54).

Besides the externalization of phosphatidylserine, cMPs carry antigens from their cell of origin (55). Therefore, MP can be characterized by Annexin V binding (which has high affinity for phosphatidylserine), and bioactive and/or biomarker molecules from their parental cells. Table 1 shows the antibodies more commonly used for MP characterization by flow cytometry, the most widely used technique for MP characterization.

cMPs contribute to both initiation and progression of CVD by increasing leukocyte activation and chemotaxis, stimulating foam cell formation by enhancing lipid and cholesterol accumulation in macrophages, and increasing endothelial activation

and platelet aggregation (53). cMPs contain lipids, proteins, microRNAs, and other noncoding RNAs, which could contribute to their functional effects (56), and may influence differential function, although further work is required to dissect the responses elicited by individual MP species in order to fully understand how they contribute to vascular inflammation and atherosclerosis.

Microparticles are investigated as candidate biomarkers for cardiometabolic diseases (Table 2). cMPs have been found increased in different CVD states and associated to several CV risk factors such as smoking, dyslipidemia, diabetes mellitus and hypertension (56,53) or essential thrombocytopenia (57). In familial hypercholesterolemia patients, platelet-derived cMPs carrying TF identified subclinical atherosclerotic plaque burden (58), and lymphocyte-derived cMPs were able to discriminate between lipid-rich and fibrous atherosclerotic plaques in the same patients (59), thus reflecting that exposure to LDL at the long term activates the vascular compartment by several mechanisms (Figure 2). Recently, platelet- and monocyte-derived cMPs have been associated with AMI severity (60) and CVD mortality (61), reflecting the sustained underlying endothelial injury and leukocyte and platelet activation in CVD progression after a MACE. In view of the literature, cMPs are emerging as novel diagnostic and prognostic biomarkers of CVD.

Table 2. Role of circulating microparticles in cardiometabolic diseases

Cell origin of cMP	Diabetes	Obesity	Hypertension	Endothelial dysfunction	Dyslipidemia	Atherosclerosis	Thrombosis
Platelets	+	+	+	+	+	+	+
Lymphocytes	+	+		+	+	+	+
Monocytes	+	+	+	+	+	+	+
Erythrocytes				+		+	+
Endothelial cells	+	+	+	+	+	+	+
Tissue Factor bearing		+	+	+	+	+	+

+ denotes a positive relationship. Empty spaces indicate lack of data. cMP indicates circulating microparticles.

4. DIETARY PATTERNS AND ATHEROSCLEROSIS

Available treatments for atherosclerosis tend to tackle its underlying causes rather than the disease directly. According to the WHO (24), the most important behavioural risk factors of heart disease and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol.

Atherosclerosis is a chronic inflammatory disease that has its onset at early stages of life. Therefore, besides postprandial and short term effects of food intake, the study of the long-term effects of food compounds and more important, dietary patterns, is required to design adequate guidelines for CVD prevention. On these grounds, dietary habits at childhood and young adulthood may potentially influence CVD risk at middle-age and in the elderly. In the CARDIA cohort, a high intake of fruit and vegetables during early adulthood, independently of age, sex and ethnicity, was inversely associated to coronary artery calcium at 20 years of follow-up, with approximately 25% of risk reduction of coronary artery calcium in subjects at the upper tertile of fruit and vegetables consumption compared to subjects at the lowest tertile of consumption, even in an adjusted model for body mass index, smoking, alcohol intake, physical activity, income, centre and education, and other potential mediators of coronary artery calcium such as blood pressure, HDL and LDL cholesterol or blood sugar levels (62). Noteworthy, coronary artery calcium has been shown to predict mortality independently of age and even minimal amounts of coronary calcification have been associated with a substantial increase in mortality compared to individuals with no coronary artery calcium (63).

It is well known that abusive or binge alcohol drinking has detrimental health effects by increasing overall mortality, cancer and cardiovascular disease. On the other hand, alcohol consumption has been consistently found to have a J-shaped association with coronary heart disease, with moderate drinkers (up to 2 drinks per day for men and up to one drink

per day for women) exhibiting a decreased risk compared to both heavy drinkers and non-drinkers. Several mechanisms have been proposed to explain the decreased risk of CVD. Besides the ApoA-I and II and HDL cholesterol raising effects, moderate alcohol consumption has been associated with lower atherosclerotic burden in the proximal aortic arch (64) and to an increased release of nitric oxide from the endothelium (65). In addition, moderate consumption of wine (especially red wine) and beer may have additional cardioprotective effects because of its high polyphenolic content, through antioxidant and anti-inflammatory mechanisms (66).

In the era of the abundance, in which metabolic epidemics such as obesity and diabetes are in continuous expansion and coexist with the social standards of fitness and healthiness, several diets have been emerged as cause or consequence of the social pressure. The next subsection and Table 3 describe the main dietary patterns consumed in the last century and their effects on atherosclerosis progression.

4.1. High fat diets

The relationship between fatty acid intake and CVD is complex, because fat intake plays a crucial role in both prevention and progression of CVD. CVD risk can be modestly reduced by decreasing the intake of saturated fatty acids (SFA) and replacing it by a combination of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). The type of unsaturated fat for this replacement is unclear; in some studies the benefits of diets rich in PUFA appear stronger than the observed for diets enriched in MUFA (67), while in others the net benefit is similar (68).

4.1.1. Western diet

A large proportion of humans have switched from a healthy diet to a Western diet. The Western diet is characterized by a high intake of processed foods (e.g., meats and refined grains), high-fat dairy products, and sugary desserts and drinks that overall is translated to a high intake of saturated and omega-6

Table 3. Dietary patterns and their main effects on cardiovascular disease and risk factors

Diet	Characteristics	Diabetes ¹	Dyslipidemia ¹	Hypertension ¹	MACE ¹
Western diet	<ul style="list-style-type: none"> • High in saturated fatty acids • High in simple sugars • Low in unsaturated fatty acids • Low in Fiber, vitamins, minerals • Low in polyphenols 	Increased	Increased	Increased	Increased
Low carbohydrate diet	<ul style="list-style-type: none"> • Low in carbohydrates and sugar • High in unsaturated fatty acids • Low in Fiber, vitamins, minerals • Low in polyphenols 	Decreased	Decreased	Neutral	— ²
Low fat diet	<ul style="list-style-type: none"> • Low in saturated fatty acids • Low in unsaturated fatty acids • High in carbohydrates • Low in sugars • High in Fiber, vitamins, minerals • High in polyphenols 	Decreased	Decreased	Decreased	Neutral ³
Vegan diet	<ul style="list-style-type: none"> • Low in saturated fatty acids • High in unsaturated fatty acids (but low in EPA and DHA) • High in carbohydrates • Low in sugars • High in Fiber, vitamins, minerals • High in polyphenols 	Decreased	Decreased	Decreased	Decreased
Mediterranean diet	<ul style="list-style-type: none"> • Low in saturated fatty acids • High in unsaturated fatty acids (including EPA and DHA) • High in carbohydrates • Low in sugars • High in Fiber, vitamins, minerals • High in polyphenols 	Decreased	Decreased	Decreased	Decreased

¹Increased reflects higher rates of intermediate CV phenotypes or higher incidence of MACE, and decreased reflects the opposite. ²Not fully explored.

³Control diet in many of the studies. MACE denotes major adverse cardiovascular events.

fatty acids (FA), low intake of omega-3 FA, and a high intake of salt and refined sugar. Several components of the Western diet can promote inflammation (69,70), and switching to that dietary pattern has been linked to metabolic diseases and atherogenesis (71) or metabolic endotoxemia (72,73). Indeed, this dietary pattern has been widely used in animal models to induce cardiometabolic diseases.

4.1.2. Low carbohydrate diets

Low carbohydrate diets have reached popularity for weight loss and weight management. Nevertheless, the low-carbohydrate diet has never been recommended in leading guidelines because a low carbohydrate diet implies a high protein and high fat diet, and therefore they have an increased risk of causing adverse effects on cardiovascular disease risk factors, mostly derived from the increased intake of saturated fats. Thus, if a low carbohydrate diet is composed by a high content of MUFA and PUFA it may not be detrimental for human health, but even cardioprotective. Low carbohydrate diets in obese patients have been shown to increase insulin sensitivity at 6 months but not after one year (74), but have been shown to increase HDL cholesterol (74–76) and decrease total and LDL cholesterol and triglyceride levels, although it is difficult to distinguish between the effects of nutrient intakes or weight lost

itself in the improvement of these CV risk factors (77). Indeed, in a recent study in overweight women, soluble (s) thrombomodulin, sE-selectin, sP-selectin, serum amyloid A and C-reactive protein were lower ($p < 0.05$) after a low carbohydrate diet compared to the normal diet, but serum lipids and apolipoproteins were not different (78). Nevertheless, long-term studies on hard endpoints are required to determine if the low intake of fruit, vegetables, and fiber consumed during a low carbohydrate diet can independently increase the risk of coronary heart disease.

4.2. Low fat diets

In contraposition to low carbohydrate diets, low fat diets are high in carbohydrates (but not simple sugars), and are also used for weight loss and management. A low fat diet decreases HDL cholesterol concentrations and increases serum triglyceride concentrations, although it also decreases diastolic blood pressure (74) and LDL cholesterol (75,76). The improvement of CVD risk factors from low fat diets may be somewhat lower than that of a low carbohydrate diet, although both diets may be hard to follow in the long term. A recent randomized clinical trial comparing low carbohydrate and low fat diets showed that both diets increased adiponectin concentrations, but only the low carbohydrate diet decreased leptin concentrations, with no net effect in any of the diets in the modulation

of the circulating levels of vascular adhesion molecule (VCAM-1), E-selectin, Interleukin (IL)-6, IL-8 or tumor necrosis factor (TNF)-alpha, although intercellular adhesion molecule (ICAM-1) decreased with the low carbohydrate diet (79).

4.3. Vegan diets

Vegetarian diets have been associated with a lower risk of coronary artery disease and stroke (80), and although high meat consumption (especially red meat) has been recently associated with increased incidence of peripheral artery disease (81), the advantage of strict vegan diet compared to ovo-lacto-vegetarian or Mediterranean diets remains unproven. A vegan diet is defined by the abstention from consuming meat and meat products, poultry, seafood and flesh from any other animal and is consequently rich in fruit and vegetables, grains and nuts (with their associated benefits for CV health, as discussed previously) but their intake of vitamin B₁₂ is usually inadequate, which is accompanied with increased plasma homocysteine levels and associated to arterial endothelial dysfunction and increased carotid intima-media thickness (82). On the other hand, vegan diets are rich in other vitamins, fiber, polyphenols and MUFA. A high MUFA intake is associated to lower concentrations of E-selectin, triglycerides and LDL cholesterol, but not to improved endothelial function (83). A diet rich in fruit and vegetables has been shown to favorably modulate intestinal microbiota to a healthier profile associated with reduced CVD risk (84), and dietary flavanols (the main group of polyphenols, present in fruit, vegetables, tea, cocoa, coffee and wine), in addition to improving cardiovascular functions, can facilitate endogenous repair mechanisms that act synergistically with current medical therapy. In CAD patients, 375 mg daily flavanols during 30 days improved endothelial function (85) by increasing flow mediated dilation, decreasing systolic blood pressure, and increasing the plasma nitrite and the mobilization of angiogenic cells to the circulation.

4.4. Mediterranean diet

The Mediterranean diet is currently considered one of the healthiest (if not the healthiest) dietary pattern for chronic disease prevention. Compelling evidence supports the beneficial effects of the Mediterranean diet for primary prevention of cardiovascular disease, by improving several cardiovascular risk factors such as diabetes, metabolic syndrome, hypertension and dyslipidemia (reviewed in 86) and intermediate biomarkers such as CRP, adiponectin or leptin (76). Indeed, the Mediterranean diet is the closest pattern to the healthy diet defined by the WHO (24). The Mediterranean diet is characterized by a high intake (at least 5 portions per day) of fruit and vegetables, a high intake of legumes, nuts, whole

grains, olive oil and aromatic spices, a moderate-high intake of fish and low meat, dairy and sweetened products consumption. Despite the high intake of fatty products such as olive oil, nuts or fatty fish, this diet is not associated to increased body weight or obesity prevalence (87).

Overall, the cardioprotective effects of the Mediterranean diet may be attributable to its high intake of MUFA, polyunsaturated omega-3 fatty acids (from plants and marine origins), vitamins, minerals, fiber and polyphenols and a low intake of simple sugars and SFA considered altogether. Therefore, besides the cardioprotective effects of a vegan-like diet, the Mediterranean diet has the additive effects of polyunsaturated omega-3 FA derived from a high intake of fatty fish (marine omega-3 FA, mainly EPA and DHA). Marine omega-3 FA have been shown to lower plasma levels of triglycerides, to reduce resting heart rate and systolic and diastolic blood pressure, to improve flow-mediated arterial dilation and to lower circulating markers of endothelial dysfunction such as E-selectin, VCAM-1, and ICAM-1 (88). Despite the beneficial effects of marine omega-3 FA in intermediate CV risk factors, their impact on the secondary prevention of CVD and their use as a supplement is currently controversial (89–91).

5. DIET AND ATHEROTHROMBOSIS

One of the strategies to reduce the risk of thrombosis is by inhibiting events involved in primary homeostasis such as decreasing cell adhesion molecule expression, inhibiting proaggregation stimuli and decreasing coagulation factors, increasing circulating inhibitors and decreasing cell activation and platelet aggregation.

Some studies have shown that diet plays a pivotal role in regulating thrombosis potential. Switching from a Western type to a Mediterranean-like or a low fat diet for 3 months has been shown to decrease the endogenous thrombin potential by 21%, independently of body weight or insulin sensitivity, and this decreased thrombogenic capacity was associated to a decreased intake of SFA and increased polyunsaturated omega-3 FA (mainly EPA) and polyphenols consumption (92). The decreased endogenous thrombin potential was associated with a parallel decrease in vitamin K-dependent factors (II, VII, IX, and X) and TF pathway inhibitor, whereas fibrinogen levels, cofactors V and VIII, antithrombin, free protein S, protein C and F1+2 levels did not vary after the 3 months of dietary changes. No differences were observed on the endogenous thrombin potential between the Mediterranean or the low fat diets because no significant differences in the percentage of macro and micronutrients were observed between diets. Indeed, the Longitudinal Investigation of Thromboembolism Etiology study observed that a

diet including more plant food and fish and less red and processed meat (both Mediterranean and low fat diets have these characteristics) is associated with a lower incidence of vascular thromboembolism (93).

As a bilateral pattern of the effects of alcohol consumption on CVD, there is consistent association between heavy alcohol consumption and a lower fibrinolytic capacity, a more procoagulant state and a higher blood viscosity (94), whereas moderate alcohol consumption is consistently associated with a decreased procoagulant state (by lowering several coagulation factors) and blood viscosity, as well as with a higher fibrinolytic capacity (increased plasminogen activator inhibitor-1/plasminogen activator -PAI-1/tPA-ratio and decreased plasma fibrinogen). In women with moderate risk for cardiovascular disease and elevated levels of C-reactive protein, moderate alcohol consumption in the context of a diet enriched in anti-inflammatory components was able to decrease both Factor VII and Factor VIII together with a prolongation of thrombin generation initiation and propagation time, but without any change in endogenous thrombin potential (95).

As reviewed (96), there are several potential food compounds that may elicit an antithrombotic effect, but the fact is that most of the studies are performed in animal or cell models with megadoses of the studied compounds. Therefore, more randomized clinical trials are required to elucidate the role of diet in thrombogenic capacity.

5.1. Diet, leukocyte activation and platelet aggregation

As mentioned previously, leukocyte activation and platelet aggregation is one of the cornerstones of atherogenesis and thrombus formation. Therefore, strategies to reduce cell activation and aggregation are of utmost importance to delay the onset and progression of atherothrombosis.

The Mediterranean diet has been shown to be cardioprotective by several mechanisms, of which decreasing leukocyte activation is one. Indeed, higher adherence to a Mediterranean diet has been associated to decreased leukocyte and platelet counts (97), and to decreased biomarkers of systemic inflammation and MACE. A Mediterranean diet enriched in olive oil for one month was also shown to decrease Nuclear Factor (NF)-kappaB activation in leukocytes (98). Moreover, three months of Mediterranean diet were shown to decrease gene expression of cyclooxygenase (COX)-2, low density lipoprotein receptor-related protein-1 and monocyte chemotactic protein-1 from monocytes (99) coordinately with decreased monocyte protein expression of CD49d and CD40, and decreased serum levels of IL6, ICAM-1, VCAM-1,

C reactive protein (100), TNFR60 and TNFR80 (101), and these effects persisted even at one year. Part of the observed effects could be attributed to polyphenols contained in this diet, because 1 month dietary interventions with different polyphenol-rich foods have been shown to decrease several inflammatory circulating and leukocyte adhesion molecules (102,103) or NF-kappaB activation in leukocytes (104). Polyphenols, and more concretely flavonoids from grape juice (or red wine) have been shown to possess antiplatelet activity in healthy subjects at middle-term, by decreasing platelet aggregation mediated by phorbol 12-myristate 13-acetate, ADP and collagen, and by increasing platelet-derived nitric oxide release (105). Interestingly, these antiplatelet effects were not observed after administration of orange or grapefruit juice (106), suggesting that (proantho)cyanidins may be responsible for these effects.

Garlic and onion have been the focus of attention because they contain sulphured compounds with potential antiplatelet activity. Nevertheless, the amount of these compounds in onion is too low to elicit any effect in a dietary context. In healthy volunteers, garlic supplementation (in dietary amounts) reduced platelet function, and this inhibitory effect was selective, affecting collagen and epinephrine but not ADP-induced aggregation (107), provably via inhibition of platelet lipooxygenase and cyclooxygenase enzymes, which in turn suppresses the production of thromboxane B2 (TXB2).

As previously shown, marine fatty acids are cardioprotective. One of the mechanisms for this effect is the incorporation of EPA and DHA to leukocyte and platelets membrane displacing arachidonic acid (a precursor of the platelet aggregator TXA2). It is known that omega-3 PUFA intake reduce serum levels of ICAM-1, P- and E-selectin, both in healthy subjects and dyslipidemic patients (108). In healthy individuals and to a lesser extent in CVD patients (109,110), omega-3 PUFA significantly reduced ADP-induced and adrenaline-induced platelet aggregation, as well as P-selectin expression on platelets and formation of platelet-monocyte aggregates after activation with 0.5. μ M ADP. In healthy ovo-lacto-vegetarian subjects 700 mg of EPA and DHA supplementation for 8 weeks, significantly reduced the maximum percentage or slope of platelet aggregation induced by ADP, epinephrin, collagen and arachidonic acid (111), but no significant changes were observed in bleeding time.

A single dose of EPA and/or DHA decreases platelet aggregability and lag time in healthy males (112). Overall, and as extensively reviewed, there is compelling evidence that marine omega-3 FA decrease platelet activation in several clinical backgrounds (34), although the effects of EPA and DHA when considered separately may be different for

healthy men and women, being EPA more favorable for men and DHA for women (113). High oleic acid intake seems to decrease postprandial Factor VIIa and Factor VIIc levels (114), even when compared to other MUFA. However, its effects at the long term remain elusive, because fasting levels of Factor VIIa and c remain unaltered after 8 weeks of a high oleic acid diet (115,116).

The antithrombotic activity of omega-3 FA has been proven beyond its antiplatelet activity. As previously mentioned, tissue factor plays a key role in thrombosis. Consuming 15 mL per day of marine oil rich in omega-3 FA (seal/cod liver oil or whale oil) during 10 weeks decreased monocyte tissue factor activity (117), and EPA + DHA supplementation for 12 weeks increased plasminogen activator inhibitor (118) in healthy volunteers. In patients with atherosclerotic disease, 3 grams daily of omega-3 FA supplementation for 16 weeks decreased prothrombin activation fragment 1 + 2 and increased plasma levels of tissue TFPI (119). Increased plasma levels of TFPI were also observed after EPA plus DHA supplementation in hypercholesterolemic patients (120). Again in healthy subjects, a Mediterranean diet decreased monocyte expression of tissue factor (121), and circulating levels of tissue factor in premenopausal women (122). In this line, we have observed that a Mediterranean diet supplemented with nuts increased TFPI expression in monocytes (99) and lowered tissue factor carrying MP from platelets (123). In contraposition, orange juice intake decreased tissue factor pathway inhibitor but not tissue factor plasma levels in healthy volunteers (124). In these subjects, orange juice decreased blood clotting time.

Legumes are rich in soluble fiber, and therefore have a lowering effect on glycemic index. Higher legume intakes are associated to lower circulating levels of soluble E-selectin, ICAM-1 and VCAM-1, C reactive protein, TNF-alpha and IL6 (125). It has been shown that oligofructose (126) and inulin (127) modulate metabolic endotoxemia by decreasing lipopolysaccharide levels in obese and diabetic patients. Besides the bioactivity of some compounds present in legumes, part of their anti-inflammatory effects may be attributed to secondary metabolites of the colonic microbiota. Along this line, a recent study has elegantly demonstrated that gut microbes can directly modulate platelet hyperreactivity and thrombosis potential *in vivo* through the generation of trimethylamine N-oxide (128), which is independently associated with incident risk for thrombotic events (129). In fact, the study about the interrelationship between diet compounds, microbiota and cardiovascular disease is a relatively novel field and of great interest, because it is currently providing new mechanisms by which some dietary patterns may be overall cardioprotective.

Noteworthy, most of the mentioned studies are performed in CVD patients or in high CV risk

subjects, which are under standard medical treatment. Therefore, these results suggest that there is still room to protect patients from excessive platelet activation besides standard antiplatelet therapy, by increasing their intake of EPA and DHA without an associated increased bleeding risk.

5.2. Diet and circulating microparticles

The relationship between diet and food components and immune/endothelial cell activation through cytokine release has been the subject of study for (a) few decades, but the study of dietary modulation of microparticle release is relatively new, and the main body of evidence relies on short-term studies analyzing only platelet or endothelial cMPs, and not cMPs from other cell origins involved in the atherosclerotic process. Despite few long-term randomized clinical trials have evaluated the effects of a dietary pattern on MP shedding, there is an increasing interest on that field. An oat-enriched diet (rich in polyphenols and with low glycemic index) during 8 weeks reduced fibrinogen- and tissue factor-positive platelet-derived MPs and monocyte-derived (CD11b⁺) cMPs in type 2 diabetic patients, in contraposition to a standard dietary advice (consisting in decreasing fat and sugar intake) which only reduced fibrinogen loaded platelet-derived MPs compared to their habitual diet (130). Overweight women after 24 weeks of low carbohydrate diet (but not less than 40 g per day) showed lower levels of CD31⁺/CD41⁻ but not CD146⁺, CD144⁺ or CD105 (endoglin)⁺ endothelial-derived cMPs compared to 24 weeks of their regular diet (78). It is plausible that results regarding the effects of a low glycemic index diet in both studies would be additive because the first study only measured platelet-derived cMPs and the latter only characterized endothelial-originated cMPs. In addition, besides the composition of diet, weight loss itself may independently provoke the inhibition of cell activation and MP release. In non-diabetic obese subjects (131), weight loss was associated with platelet (CD41⁺) cMPs decreased release, and obese women after 3 months under a very low calorie diet showed decreased release of platelet (GPIb⁺)- and leukocyte (CD11a⁺ and CD4⁺)-derived cMPs, but not from erythrocytes or endothelial cells (132).

The impact of the quantity and quality of dietary fats (saturated, monounsaturated and polyunsaturated) on circulating levels of MP deserves some discussion. While high saturated fat meals increase platelet and endothelial MP shedding (133,134), at least in the postprandial situation (135), and EPA decreases MP activity but not CD41⁺ platelet-derived MP shedding (112), the roles of long-term intake of omega-6 and omega-3 FA on MP shedding are unclear, and some studies have found controversial results. A 8 weeks fish-oil supplementation (900 mg per day EPA and 600 mg per day DHA) decreased the

number of endothelial (CD31⁺/CD42b⁻)-cMPs, but not platelet (CD31⁺/CD42b⁺)-derived cMPs in moderate CV risk subjects compared to a corn oil placebo (136). In healthy subjects, EPA and DHA supplementation (1200 mg per day) for 4 weeks had no effect on CD36⁺ (a scavenger receptor for both native and oxidized lipoproteins expressed in several cell types) or platelet-derived (CD41⁺) MP shedding (137), possibly because of the low degree of cell activation of healthy subjects. Nevertheless, the effects on marine omega-3 FA on MP shedding may differ in secondary CV prevention, because in patients with an AMI, 12 weeks of omega-3 FA supplementation (4.3 g of EPA and DHA per day) decreased platelet (CD61⁺) and monocyte (CD14⁺) but not endothelial (CD62E⁺)-derived or tissue factor (CD142) positive cMPs compared to (non-extra virgin) olive oil (138,89).

Besides their anti-inflammatory and antioxidant effects, the impact of polyphenols in MP shedding has been also the subject of study. In CAD patients, 375 mg of cocoa flavonols daily during 30 days decreased endothelial CD31⁺/CD41⁻ and CD144⁺ but not platelet CD41⁺ MP shedding (139). About 13 grams per day of cocoa powder during a month decrease endothelial-derived cMP in overweight or obese women (140), but not in normal-weight women. Noteworthy, overweight and obese women showed higher baseline levels of cMP from endothelial origin (CD144⁺/CD42a⁺/CD45⁻).

The Mediterranean diet, which is rich in polyphenols, has been shown to be cardioprotective through several mechanisms, including MP shedding. Elderly subjects at middle-high cardiovascular risk after 4 weeks of a Mediterranean Diet enriched in extra virgin olive oil showed decreased CD31⁺ and CD144⁺/CD62E⁺ MP shedding from endothelial cells (141). In high cardiovascular risk patients we have observed that a Mediterranean diet supplemented with extra virgin olive oil decreased MP shedding from activated platelets (PAC-1⁺), lymphocytes (CD3⁺/CD45⁺) and smooth muscle cells (SMA- α ⁺) after one year of intervention (142). The differences observed in both studies may be partially provoked by the different antibodies used to quantify endothelial MPs (CD31 and CD144 versus CD146) and also to slight differences in the macronutrient compositions of both diets, although differences at short or long term may not also be discarded. In parallel, one year intervention with a Mediterranean diet supplemented with nuts hampered MP shedding from platelets (PAC-1⁺, CD61⁺, CD142⁺/CD61⁺ and CD62P⁺), endothelial cells (CD146⁺) and activated leukocytes (CD63⁺ and CD11a⁺) (123). Nevertheless, these results were observed only in those patients who were not to suffer a future MACE, while in patients who suffered a MACE at the end of the study follow-up (which account for about 3% of the original cohort), these cMPs were increased at one

year of intervention. These results suggest a heretofore unrecognized reason why individual variability may occur in response to dietary habits, which may be partially solved through nutrigenomic studies.

Overall, the effects of food compounds and dietary patterns on MP shedding in different physiopathological situations are summarized in Figure 3. A high intake of SFA is associated to increased MP shedding from platelets and endothelial cells, while a high intake of omega-3, polyphenols and polyphenol-rich products such as extra virgin olive oil or nuts is associated to (with) decreased MP shedding from several cell types. The effects of diet in MP shedding are an emergent area of interest, and will unveil novel mechanisms by which food intake elicits protective or detrimental effects on CVD development and progression.

5.3. Diet and MACE

A lot of efforts have been invested in measuring the effects of diet interventions in intermediate biomarkers of CVD, but public health policies should be directed towards reducing the incidence of MACE. To preserve cardiovascular health, the importance of maintaining a high intake of fruit and vegetables has been widely accepted. A meta-analysis of observational cohorts found that each additional serving of fruit and vegetables was associated with a risk reduction of 4% for a coronary heart disease event (143). In the same line, in the National Health and Nutritional Examination Survey, subjects who consume fruit and vegetables three or more times per day had about 37% lower risk of CVD mortality compared to individuals who reported consuming fruit and vegetables less than 1 time per day (144). Indeed, the American College of Cardiology/American Heart Association lifestyle guidelines encourage a diet that includes a high intake of fruit and vegetables as a Class 1-Grade A recommendation to lower cardiovascular risk (145) in the grounds that a long term (5 years) intervention with Mediterranean diet -rich in fruit and vegetables- reduced the incidence of MACE by 30% (68), principally by reducing stroke incidence, as supported by other groups (146). The mechanisms by which the Mediterranean diet decreases the risk of stroke in a superior level than decreases the risk of AMI remains unrevealed.

Consumption of fish or fish oil significantly reduces CHD mortality, including fatal myocardial infarction and sudden cardiac death, in populations with and without established CVD, mainly by reducing ischemia-related cardiac death (88). A pooled analysis of 19 cohort studies showed that a 1-SD increase in omega-3 FA plasma levels (sum of EPA, DPA and DHA), was associated with an approximately 11% lower risk of fatal CHD but not with non fatal MI (89),

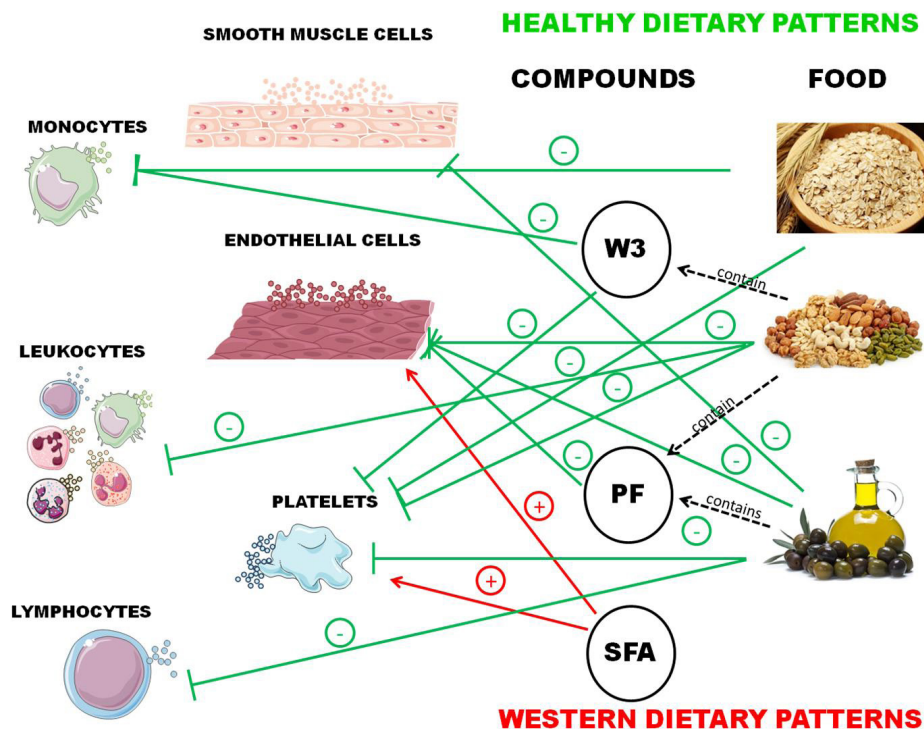


Figure 3. Effects of foods and food compounds on circulating microparticles. While a high intake of saturated fatty acids (SFA) increase circulating levels of platelet- and endothelial-derived microparticles, foods and food compounds characteristics of healthy dietary patterns such as the Mediterranean diet or a Vegetarian diet have been shown to reduce levels of circulating microparticles from several cell origins. SFA indicates saturated fatty acids; W3, omega-3 marine fatty acids; and PF, polyphenols.

although not all randomized trials observed a reduction on MACE risk (147).

Several scores of healthy dietary habits have been elaborated for and related to a decreased MACE incidence, because interventional trials evaluating hard endpoints are as necessary as expensive and difficult to perform. The Alternative Healthy Eating Index 2010 (AHEI2010) correlated inversely with 26% lower cardiovascular mortality comparing quintiles 1 and 5 of score in patients who had suffered a previous AMI (148), and a 1 point increase in a 15-points Mediterranean diet score in patients with a baseline score of 12 points with a previous MACE was associated to a 5 percent reduction of MACE risk (149). Interestingly, in this study, a Western diet score was not associated to increased (or decreased) risk of MACE, although a high intake (more than 15 percent of the total energy intake) of sucrose (simple sugar, characteristic from the western type diet) in a Swedish cohort was associated to 37 percent increased risk of coronary events compared to subjects consuming less than 5 percent of the total energy in the form of sucrose (150). A reduced-salt Japanese diet score was also associated to decreased risk of CVD mortality and stroke (151). An unhealthy diet, defined by several parameters but low intake of fruit and vegetables as the main contributors, accounted for 5–70% of

cardiometabolic deaths in Middle East countries (152). Vegetarian diets showed no differences in incidence and/or mortality from cardiovascular diseases compared to omnivore diets apart from ischemic heart disease, which showed 25% lower relative risk compared to omnivore diets (153). Nevertheless, high intake of fruit and vegetables rich in antioxidants (vitamins and polyphenols) decreases intermediate biomarkers of CVD risk, as previously mentioned. On the other hand, low plasma levels of vitamin C are associated with severity of atherosclerosis and predict unstable coronary syndrome (154,155). The benefits of supplementation of both vitamins have not been validated in randomized controlled trials (156), and considering that vitamins in excess have been associated to several chronic diseases, caution should be taken with vitamin supplements.

6. CONCLUSIONS AND FUTURE PERSPECTIVES

The study of the effects of diet in CVD is complex. However, worldwide compelling evidence shows that healthy dietary patterns, rich in fruit and vegetables with moderate consumption of fish, may help to decrease CVD incidence and mortality. Identifying novel risk factors and biomarkers for CVD, such as cMPs, and understanding their interaction

with food compounds will help in developing novel preventive and therapeutic measures. To develop preventive dietary policies, although some useful scores have been proposed (157), future efforts may be focused on defining the normal range of multiple parameters related to cell activation, oxidation and low grade inflammation and its associated CVD risk. Finally, it is worth mentioning that in the near future, the “omic” approach will provide reliable biomarkers in order to evaluate real food intake, and to avoid food-related misconceptions (158,159), also unveiling new insights into molecules and mechanisms of which we are not yet aware, to achieve a better understanding of the complex relationship between diet and CVD.

7. ACKNOWLEDGMENTS

This work was supported by grants from Spanish Ministry of Economy and Competitiveness of Science (SAF2016–76819-R to L.B.); Institute of Health Carlos III, ISCIII (TERCEL - RD16/0011/0018 and CiberCV- CB16/11/0041 to L.B.); FEDER “Una Manera de Hacer Europa”; the Secretary of University and Research, Department of Economy and Knowledge of the Government of Catalonia (2014SGR1303 to L.B.); and “CERCA Programme/Generalitat de Catalunya” Spain. We thank FIC-Fundacion Jesús Serra, Barcelona, Spain, for their continuous support. G C-B is a Juan de la Cierva- Incorporación Postdoctoral Fellow (IJCI-2015–26358) from the Spanish Ministry of Economy and Competitiveness (MINECO), Spain.

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Abbreviations: ADP: adenosine diphosphate; AMI: acute myocardial infarction; cMPs: circulating microparticles; COX: cyclooxygenase; CVD: cardiovascular disease; FA: fatty acids; HDL: high-density lipoprotein; ICAM-1: intercellular adhesion molecule-1; IL: interleukin; LDL: low-density lipoprotein; MACE: major adverse cardiovascular event; MetS: metabolic syndrome; MUFA: monounsaturated fatty acids; NF: nuclear factor; NO: nitric oxide; PUFA: polyunsaturated fatty acids; ROS: reactive oxygen species; SFA: saturated fatty acids; TFPI: tissue factor pathway inhibitor; TNF: tumor necrosis factor; TX: thromboxane; PAI-1/tPA: plasminogen activator inhibitor-1/ plasminogen activator; VCAM-1: vascular adhesion molecule-1; vWF: von Willebrand factor.

Key Words: Atherosclerosis, Thrombosis, Platelets, Circulating microparticles, Mediterranean diet, Polyphenols, Unsaturated fatty acids, Review

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